Cortical slow wave activity correlates with striatal synaptic strength in normal but not in Parkinsonian rats

Galati, Salvatore ; Song, Wei ; Orban, Gergely ; Luft, Andreas R ; Kaelin-Lang, Alain

Abstract: Urethane-induced cortical slow wave activity (SWA) spreads into the basal ganglia in dopamine (DA)-depleted rat models of Parkinson's disease (PD). During physiological sleep, SWA is powerfully expressed at the beginning of night and progressively reduced during sleep-time reflecting the sleep need. However, its underlying slow oscillations may contribute directly to modulate cortical plasticity. In order to determine the impact of the SWA on synaptic strength and its interplay with DA, we simultaneously recorded the electrocorticogram (ECoG) and the corticocortical- and corticostriatal-evoked potentials (CC-EPs, CS-EPs) during eight hours of robust urethane-induced SWA in both normal and PD animals. A subgroup of PD rats was assessed with repetitive apomorphine (APO) administrations. Normal animals showed a progressive reduction of SWA power during urethane-induced SWA. Compared to normal animals, PD animals showed lower SWA power at the start of anesthesia without a significant reduction over time. Accordingly, synaptic strength measured by CC- and CS-EP amplitudes decreased in normal but not in Parkinsonian rats. The PD animals treated with APO showed a CS-EP amplitude reduction comparable to normal animals. Interestingly, SWA power directly correlated with CS-EP amplitude in normal animals. These data support the hypothesis that cortical SWA is directly associated with the regulation of synaptic efficacy in which DA exerts a crucial role.

DOI: https://doi.org/10.1016/j.expneurol.2017.12.004

Cortical slow wave activity correlates with striatal synaptic strength in normal but not in 6-OHDA Parkinsonian rats

Salvatore Galati*[1], Song Wei[1], Gergely Orban[1], Andreas R. Luft[2,3], Alain Kaelin-Lang[1]

1. Laboratory for Biomedical Neurosciences (LBN), Neurocenter of Southern Switzerland, Lugano, Switzerland

2. Vascular Neurology and Rehabilitation, Department of Neurology, University Hospital of Zurich, Zurich, Switzerland

3. Cereneo Center for Neurology and Rehabilitation, Vitznau, Switzerland

* Correspondence to:

Dr. Salvatore Galati, Basal Ganglia Research Group Leader, Laboratory for Biomedical Neurosciences (LBN), Neurocenter of Southern Switzerland, Via Tesserete 46, 6903 Lugano.

Tel +41 (0)91 8116921

Fax +41 (0)91 8116915

email: salvatore.galati@eoc.ch

Running title: Urethane-induced synaptic downscaling.

Abstract

Urethane-induced cortical slow wave activity (SWA) spreads into the basal ganglia in dopamine (DA)-depleted 6-OHDA rat models of Parkinson's disease (PD). The cortical SWA during physiological sleep reflects the sleep need being powerfully expressed at the beginning and progressively reduced during sleep-time. It has been postulated that the SWA during sleep is able to modulate cortical plasticity.

In order to determinate the impact of the SWA on synaptic strength and its interplay with DA, we simultaneously recorded the electrocorticogram (ECoG) and the corticocortical- and corticostriatal-evoked potentials (CC-EPs, CS-EPs) during eight hours of robust urethane-induced SWA in both normal and PD animals. A subgroup of PD rats was assessed with repetitive apomorphine (APO) administrations.

Normal animals showed a progressive reduction of SWA power during urethane-induced SWA. Compared to normal animals, PD animals showed lower SWA power at the start of anesthesia without a significant reduction over time. Accordingly, synaptic strength measured by CC- and CS-EP amplitudes decreased in normal but not in Parkinsonian rats. The PD animals treated with APO showed a CS-EP amplitude reduction comparable to normal animals. Interestingly, SWA power directly correlated with CS-EP amplitude in normal animals.

These data support the hypothesis that cortical SWA is directly associated with the regulation of synaptic efficacy in which DA exerts a crucial role.

Keywords: Sleep benefit, Parkinson’s disease, dopamine, slow wave activity, synaptic homeostasis hypothesis.

Statement of significance

The present manuscript demonstrates that the SWA is able to downscale synapses when artificially induced with urethane. Furthermore, DA exerts a permissive role on SWA function and since it is clearly reduced in PD animals at the onset of sleep, on cortical plasticity too.

The translational implication of the present results is that the alternating administration of DA-ergic treatment during the daytime and the complete washout during nighttime could be responsible for the instauration of aberrant plasticity as observed in advanced stages of PD patients. Clinical counterparts of the present data arise from the observation that continuous antiparkinsonian treatments, such as deep brain stimulation are able to improve the levodopa-induced dyskinesia that are linked to aberrant plasticity.

The clinical message could be relevant on the management of PD therapy, suggesting that DA-ergic treatment could be initiated at PD onset with a preference for long lasting molecules able to act also during nighttime when SWA plays a crucial role in homeostatic processes.

Introduction

SWA consists of a slow electroencephalogram (EEG) oscillation ranging between 0.1 and 4 Hz, in which the depolarized up-state is sustained by the activity of cortical neurons, whilst the hyperpolarized down-state is characterized by widespread cortical neuronal silence.[1] The number of neurons synchronously engaged in the depolarized and hyperpolarized state determines the amplitude of the SWA.[1] Urethane anesthesia is able to induce cortical SWA which is indistinguishable from the physiological SWA of non-rapid eye movement (NREM) [2,3] and therefore this anesthetic has been used as a model to investigate central mechanisms underlying sleep.[4–6] Particularly, during SWA, cortical neurons show the same electrophysiological characteristics in both urethane anesthesia and NREM sleep.[2,7] Thus, it has been postulated that urethane most likely promotes unconsciousness by activating the brain mechanisms involved in natural sleep.[7] In animal models of Parkinson's disease (PD), urethane-induced cortical SWA powerfully spreads within the basal ganglia [8–10] and this evidence has been linked to an excessive synchronization between cortex and basal ganglia in the dopamine (DA) depleted state[11] interfering with motor program selection.

Indeed, DA depletion also alters the corticostriatal synaptic plasticity, as has emerged in *in vitro* studies [12] but also in *in vivo* experiments.[13] Of note, the corticostriatal-evoked potentials (CS-EPs) change their response in relation to both sleep and DA denervation. [14–18]

In physiological conditions, the amplitude of the SWA during NREM not only reflects the sleep needed [19] but could also contribute to the modulation of synaptic plasticity.[19] Accordingly, recent evidence has demonstrated that sleep shrinks synapses of sensory-motor cortex in terms of synaptic interface and volume.[20]

Indeed, the cortical sleep-like oscillations induced through electrical stimulation of the medial lemniscus modulates glutamatergic synaptic plasticity.[21] Accordingly, the corticocortical-evoked potentials (CC-EPs), representative of the global cortical synaptic strength,[22] increase with time spent awake and decrease with time spent asleep.[23]

Therefore, SWA might play a dual role in a feedback control loop in which synapses with stronger efficiency produce a larger SWA amplitude that subsequently operates an homeostatic [24] reduction of synaptic strength.[25] The long-lasting depression of excitatory postsynaptic potentials could be triggered by the oscillatory nature of the SWA.[24,26]

Given these premises, we investigate the effect and time-course of long-lasting urethane-induced SWA on synaptic transmission measured by an input-output (I-O) curve of the CC-EP and CS-EP. Subsequently, we explore the impact of chronic DA depletion and DAergic treatment on these evoked responses and on SWA changes.

We hypothesize that dopaminergic denervation strongly affects SWA and the related evoked potentials. We used the evoked field potentials because they are a reliable tool for studying synaptic communication and especially excitatory post-synaptic potentials *in vivo*.[27] Moreover, the monosynaptic connection of the CC-EP and CS-EP lead to a straightforward identification of the evoked response [23,27,28] that is linked to synaptic strength.[22,23,27] We focused our study on frontal motor cortex evoked responses because the most substantial increase after prolonged wakefulness, as measured by SWA power, has been described within this region. [20,29]

Methods

Animals

Experimental procedures were carried out on 16 adult male Sprague-Dawley rats (Harlan, Udine, Italy) that weighed between 170-200 g, corresponding to six weeks of age. The study was conducted at the Laboratory for Biomedical Neurosciences (LBN) in compliance with Swiss laws on animal experimentation and the National Institute of Health`s Guide for the Care and Use of Laboratory Animals. The study was approved by Ticino veterinary authorities. All procedures were performed with the intention of minimizing animal discomfort and stress. The rats were kept on a regular light-dark cycle (lights on at 08:00am, lights off at 08.00pm; room temperature 20-22°C;) and were given food (Harlan RM Diet, Udine, Italy) and water *ad libitum*. The study design and the experimental procedures are depicted in Figure 1A,B.

Unilateral Parkinsonian 6-OHDA-lesioned model

A unilateral (right hemisphere) DA denervation was performed on 10 rats according to a standard protocol [28,30,31]. The rats were anaesthetized with 1.5-2.5% isoflurane in oxygen and were mounted on a stereotaxic instrument (Stoelting Co., Wheat Lane, Wood Dale, IL, USA). Body temperature was maintained at 37°-38° C with a heating pad (Stoelting Co., Wheat Lane, Wood Dale, IL, USA) that was placed beneath the animal. After a subcutaneous injection of the local anesthetic lidocaine, a midline scalp incision was made and a hole (Ø ~1.0 mm) was drilled in the skull on the right side. The neurotoxin (30 mM saline solution of 6-OHDA containing 0.03% of ascorbic acid) was injected into the medial forebrain bundle (MFB; coordinates: 4.0 mm posterior of the bregma, 1.3 mm laterally of the midline and 7.0 mm beneath the cortical surface). Injections of 3 µl of 6-OHDA were administered through a 30-gauge cannula connected to a 10 µl Hamilton syringe over a period of 3 minutes. The injection of the neurotoxin was preceded by a bolus of desipramine (25 mg/kg, i.p.) in order to minimize the uptake of 6-OHDA by noradrenergic neurons.

Tyrosine hydroxylase immunohistochemistry

After completing the recordings, the 10 rats were sacrificed with an i.p. overdose of urethane. The brains were removed immediately, and then frozen at -20°C. We measured the degree of dopaminergic damage within the substantia nigra compacta (SNc), the ventral tegmental area (VTA) and the ipsilateral striatum by tyrosine hydroxylase (TH) immunostaining (Figure 1C), as reported elsewhere.[18,28,30,32] Briefly, coronal brain 40-µm-thick sections were cut with an Oxford vibratome across the entire rostrocaudal extent of the striatum and midbrain. Free-floating sections were washed three times with Tris-buffered saline, pH 7.4, and endogenous peroxidase activity was inactivated by Tris-buffered saline containing 2% H2O2. The sections were rinsed with Tris-buffered saline (0.1% Triton X-100 and 2% normal goat serum) and incubated with 2% normal goat serum followed by overnight incubation at 4°C with mouse anti-TH primary antibodies (1:2000; anti-TH Chemicon, Millipore). Primary antibodies were detected using a secondary antibody (goat anti mouse IR800, Rockland, Gilbertsville). Sections were then rinsed with Tris-buffered saline, mounted on gelatin-coated slides, dehydrated, and cover-slipped with Permount, observed and photographed with a light microscope. All histological samples showed absence of TH reaction with a preserved VTA ipsilateral to 6-OHDA injection.

Electrophysiological recordings

The electrophysiological recordings were performed under urethane anesthesia maintaining the body temperature at 37°-38° C with a heating pad (Stoelting Co., Wheat Lane, Wood Dale, IL, USA) that was placed beneath the animal. We continuously recorded the cortical surface EEG (ECoG) and performed the input-output (I-O) curves of the CC-EP and the CS-EP starting at the same hour in the morning, and then every 2 hours during eight consecutive hours in normal (n = 6) and untreated Parkinsonian rats (n = 5; Figure 1A,B). An additional group of Parkinsonian animals (n = 5) was injected with apomorphine (APO, 0.05 mg/kg, s.c.) 10 minutes before each I-O curve. Two 1mm diameter golden screw electrodes (gold plated screws, conical cross S1, Svenska Dentorama AB)

were implanted in the frontal right and left cortices (coordinates: 2 mm anterior of the bregma, 3 mm laterally to the midline) under urethane anesthesia.[31] Furthermore, a tungsten recording electrode with an impedance of ~0.1 MΩ (TM33B01, World Precision Instruments, FL, USA) was implanted into the striatum (coordinates: 0.48 mm anterior of the bregma, 3.5 mm lateral to the midline and 4 mm ventral to the cortical surface).[18] One reference epidural screw electrode was placed over the right cerebellar hemisphere.

Data acquisition and stimulation protocol

The signal was amplified (gain: 1K, band-pass filter: 1 Hz – 3 kHz; Iso-DAM8, World Precision Instruments, FL, USA), digitized (sampling rate: 10 kHz; Cambridge Electronic Devices, Cambridge, UK), and stored in a computer for offline analysis. The stimulation protocol consisted of constant current pulses with the following parameters: 0.3 ms duration at 0.1 Hz, 1–10 mA (AM-systems, Isolated Pulse Stimulator, Model 2100). The I-O characteristics of synaptically evoked intercallosal and cortico-striatal field potential responses were determined by increasing stimulation intensity stepwise from threshold to saturating response (from 1 to 10 mA). For each stimulation intensity, amplitudes of ten evoked responses were averaged. We sampled the CC-EP and the CS-EP every two hours for a total of 8 hours of recordings: 10.00am, 12.00pm, 2.00pm, 4.00pm (Fig. 1A).

The response of the CC- and the CS-EP consisted of a complex stereotyped sequence of field potentials, which typically consisted of a positive-negative-positive (P1-N2-P2, Fig. 2D) waveform. P1 amplitude augmented with increased stimulating current.[27,33] An earlier negative component (N1) as described elsewhere[33], was likely masked by the stimulation artifact. The amplitude of the evoked local field potentials was determined as the voltage difference between the N2 peak and the subsequent P2 peak.

Changes in the current required for an evoked response, i.e. the excitability, were determined by plotting the amplitude and the slope values of the CC-EP or the CS-EP during each current step vs. the intensity of the current injected).

Data and statistical analysis

In order to demonstrate the long-lasting stability of the urethane-induced SWA, we measured the content of dominant power during the all ECoG recordings using a fast Fourier transform (FFT) algorithm (SUDSA22 script, Spike2 software; CED). The same software calculated the total power of delta (0.1-4 Hz), theta (5-7 Hz), beta (8-13 Hz) and gamma (15-35 Hz) for 10 minutes before each stimulation protocol (10.00am, 12.00pm, 02.00pm, 04.00pm). To detect changes in the total power at the different hours, we used a non-parametric repeated-measures two-way ANOVA followed by a Dunnett's multiple comparisons test.

Data relating the EP amplitude to different stimulus strengths were first analyzed across all groups with an ANOVA for repeated measures which demonstrated, as expected, an overall highly significant effect of stimulus strength on the amplitude but no significant difference between the three groups in the I-O curves (data not shown, SPSS for Windows v9, SPSS; Chicago, IL). Therefore, we expressed all amplitude and slope values as percentages of the plateau at baseline (set to 100%) and analyzed the time course of the I-O curves by nonlinear least squares curve fitting using the GraphPad Prism, version 6.01 (GraphPad Software, Inc.). The individual curves at different intervals (10.00am, 12.00pm, 2.00pm, and 4.00pm) describing the relationship between output spike amplitude/slope (y) and stimulus intensity (x) at each stage of the cycle were fitted to a sigmoidal four-parameter Boltzmann function:

$$Y=D+(A-D)/(1+\exp((V50-X)/B))$$

A and D are the expected maximum and minimum responses, respectively. B is the Hill slope coefficient, and V50 is the stimulus strength expected to generate a half-maximal amplitude/slope response of the EP. The amplitudes and slope recorded at the highest stimulation intensities were not significantly different across time-points (data not shown) within each group and the maximum A was thus set to 100%. Constrained curve fitting (between 0% and 100%) and extra sum-of-squares F

test were then utilized to test the significance of differences between the V50 from the three groups of animals.

Furthermore, the four V50 values calculated at each hour (10.00am, 12.00pm, 02.00pm, 04.00pm) were compared by a repeated-measure one-way ANOVA followed by a Dunnett's multiple comparisons test.

Results

Urethane caused a robust cortical slow wave activity

In all animals (n = 16), we recorded the ECoG activity from both hemispheres. As previously described [1,2,28,32] the urethane anesthesia caused robust and persistent SWA of 0.1-4.0 Hz with large amplitude. Furthermore, the ECoG spectral analysis demonstrated that the dominant frequency remained within the SWA range (< 3 Hz, Fig. 2A) throughout the time course of the anesthesia in all animal groups, attesting the stability of the anesthesia.

Urethane decreased the total power in slow wave activity range only in normal animals

Although the SWA range (0.1-4 Hz) was the dominant frequency, its total power changed significantly during the 8 hours of urethane anesthesia. In fact, the total power of the SWA decreased significantly during the four different hours (10.00am, 12.00pm, 02.00pm, 04.00pm) and between the three groups of rats (ANOVA, $F_{3, 39} = 5.26$; P = 0.0038; $F_{2, 13} = 4.74$; $P = 0.0283$, respectively). However, only normal animals showed a significant decrease of the total power between 10.00 am and the following hours (n = 6; $P < 0.05$; 10.00am vs. 12.00pm: mean difference 0.062; 95% CI of difference 0.027 to 0.097, 10.00am vs. 02.00pm: mean difference 0.068, 95% CI of difference 0.033 to 0.103; 10.00am vs. 04.00pm: mean difference 0.068, 95% CI of difference 0.033 to 0.103; Fig. 2B). In Parkinsonian untreated (n = 5) and treated with APO (n = 5) animals, there were no clear changes ($P > 0.05$) of the SWA total power (Fig.2B). Of note, the total delta power at 10.00am in normal rats (n = 6) was significantly higher than in non-treated (6-OHDA, n = 5) and treated (6-OHDA+APO, n =5) Parkinsonian animals ($P < 0.001$, Control vs. 6-OHDA: mean difference 0.079; 95% CI of difference 0.040 to 0.118, Control vs. 6-OHDA+APO: mean difference 0.053, 95% CI of difference 0.014 to 0.092; Fig. 2C).

The total power of theta (5-7 Hz), beta (8-13 Hz) and gamma (15-35 Hz) remained unchanged during the time course in all groups (data not shown).

Urethane changed the corticocortical- and corticostriatal-evoked potentials excitability in normal rats

In all animals (n = 16), the CC-EP and CS-EP amplitudes increased as a function of stimulus intensity, best fitted with a sigmoidal relationship. The four I-O curves of both the CC-EP and the CS-EP amplitude performed every 2 hours (10.00am, 12.00pm, 2.00pm, and 4.00pm) progressively shifted with time of recording (Fig. 3A,B upper plots). Non-linear regression analysis of the CC-EP and CS-EP amplitude demonstrated that best fitted curves were significantly different (CC-EP: $F_{6,32}$= 23.10, P <0.001; CS-EP: $F_{6,32}$= 8.702, P <0.001; Fig. 3A,B upper plots). The V50, that is the stimulus strength able to give a half-maximal amplitude response of the EP, was lower for the I-O curves at 10.00am (Fig. 3A,B lower plots) suggesting a stronger synaptic coupling (i.e. synaptic efficiency) because low current strength is required to drive both the CC- and the CS-EP (Table1 and Table2). The fit of the subsequent I-O curves (12.00pm, 2.00pm, and 4.00pm) showed a V50 increase attesting to a reduction of the excitability of the evoked responses (Table1 and Table2; Fig. 3A,B lower plots). The four V50 values of the CC- and the CS-EP obtained at 10.00am, 12.00pm, 2.00pm, and 4.00pm were significantly different (CC-EP: $F_{1.2,6.2}$= 6.058, P <0.05; CS-EP: $F_{1.7,8.7}$= 4.914, P <0.05; Fig. 3A,B). Multiple comparison analysis showed a statistical difference in both the CC- and the CS-EP at 4.00pm versus 10.00am (Fig. 3A,B lower plots).

Effect of urethane on the corticocortical- and corticostriatal-evoked potentials excitability in Parkisonian rats

In 6-OHDA animals (n = 5), non-linear regression analysis of the CS-EP amplitude demonstrated that best-fitted I-O curves were not significantly different, leading to a shared curve for all four hours ($F_{6,192}$= 0.410, P >0.05; Fig. 4 upper plot). The V50 values were not significantly different from each other ($F_{1.1,4.4}$= 0.793, P = 0.43; Fig. 4 lower plot).

In the Parkinsonian animals treated with APO (n = 5), non-linear regression analysis of the CS-EP amplitude demonstrated that the best-fitted I-O curves were significantly different ($F_{6,192}$= 2.411, P

<0.05; Fig. 5 upper plot). In contrast to what was observed in control animals, the I-O curve at 2.00pm was the most leftward followed by the I-O curves at 12.00pm, 4.00pm, and 10.00am (Fig. 5 upper plot, Table 3). The four V50 values of the CS-EP obtained at the 10.00am, 12.00pm, 2.00pm, and 4.00pm were not significantly different ($F_{2.2,9.0}$= 0.998, P = 0.41; Fig. 5 lower plot).

In both Parkinsonian treated and non-treated rats the CC-EP were unaffected by the time spent in urethane anesthesia (data not shown).

Changes of the total power in the slow wave activity range correlate with the changes of corticostriatal-evoked potentials excitability only in naïve animals

If urethane-induced SWA drives changes of corticostriatal excitability, the evoked response would be expected to vary with the magnitude of the cortical SWA. Hence, the relationship between SWA total power and CS-EP V50 was investigated. A significant positive correlation was found between the percentage of variation (4.00pm vs 10.00am) of both SWA total power and the V50 of the CS-EP only in control animals (r = 0.91, R^2 = 0.835, p < 0.01, n = 6; Pearson's correlation, Fig. 6A). On the other hand, no correlation was observed in either 6-OHDA or 6-OHDA + APO animals (Fig. 6B,C)

.

Discussion

Recent evidence suggest that the dopaminergic system is a strong modulator of cortical activity with regard to sleep-wake states. A recent study in humans with lower activity of DA reuptake transporter shows altered SWA after sleep deprivation.[34] Indeed, the SWA is involved in pathophysiological aspects of PD.

The present findings are obtained from urethane-induced long-lasting cortical SWA[1,2] that is able to drive a cortical pattern comparable to natural sleep. [4,6,7]

Herein, the main message is twofold: (i) SWA promotes changes in synaptic efficiency in both cortex and striatum, exclusively in a DA preserved circuitry; (ii) Parkinsonian animals present lower SWA

total power at the beginning of the urethane-induced SWA. Particularly, we observed a progressive decrease of the SWA total power along with the cortico-cortical and cortico-striatal excitability measured trough EP responses.

Similarly, in normal subjects the total power of SWA during NREM sleep is highest at sleep onset and decreases proportionally during time spent asleep.[36] Although we did not examine the underlying mechanisms by which synaptic strength declines, we found that it depended on the time spent in urethane-induced SWA and on the integrity of the DAergic system.

The evoked local field responses are a reliable readout of synaptic transmission suitable for our *in vivo* study.[27] The SWA as well as the amplitude of the evoked responses, reflecting a sub-threshold phenomenon, are both directly related to the number of the neurons firing in unison according to the number, the strength, and the distribution of the synaptic bouton.[25,27,37] In humans, the SWA increases in parallel with the cortical synaptic strength promoted by learning.[38,39] Similarly, in experimental animals the infusion of BDNF increased the cortical synaptic strength and the SWA amplitude.[40] Furthermore, the CC-EPs in rats are higher after wakefulness and lower after sleep period [23] attesting to synaptic upscaling during awake state and downscaling during sleep. Our experimental data sustain the SHY postulating that the SWA is an index of cortical synaptic strength as well as a measure of sleep mediated synaptic scaling.

The further role of the SWA as a contributor for synaptic downscaling remains without direct evidence but postulated[19] by the SHY. The SHY holds that the underlying slow oscillations of the SWA would promote changes of synaptic efficiency due to the sustained cortical firing during the depolarized up state[1] able to depress synaptic strength as inferred in a computational model analysis.[26] Here we show that SWA changes are directly correlated with changes in corticostriatal synaptic strength in healthy animals.

We observed that the total dominant frequency of the ECoG was constantly below 3 Hz during the eight hours of urethane anesthesia, although the total power of the same SWA showed a significant

reduction during the four periods of analysis without changes in other frequency bands. These observations could be explained by a sustained urethane-induced cortical SWA that promotes a reduction of its power over time. The negligible effect on the power of other frequency ranges (theta, beta and gamma) supports the view that this effect is specific to SWA. We observed that only the delta, i.e. the SWA power, selectively reduced during the eight hours of robust SWA. This supports a specific SWA mediated effect instead of an unspecific effect of urethane on cortical function. These data are reinforced by the findings on Parkinsonian rats that do not show a clear decay of the delta total power or changes of synaptic efficiency.

An addition, a major result of our study was the higher SWA total power observed in healthy rats at the end of the wake time in comparison to Parkinsonian rats even after dopaminergic treatment. These findings suggest an impairment of synaptic strength build-up during the awake period in DA depleted animals. Indeed, Parkinsonian animals manifest an impairment of plasticity that is restored after levodopa treatment at least at striatal level.[12] Of note, TMS studies have shown a similar impaired cortical plasticity also in drug naïve PD patients.[41]

The lateralized 6-OHDA model could provide for misleading interpretation of the inter-hemisphere study. However, an increased nigro-striatal inter-hemisphere projection has been described after unilateral DA depletion.[42] With this limitation in mind, in hemi-parkinsonian animals both the SWA power (recorded in the normal hemisphere) as well as the CC-EP remained unchanged during the time spent in urethane anesthesia. At cortical level, APO treatment did not restore this effect. For CS-EP curves, however, DA depletion removed the reduction of excitability and this was restored by the DAagonist (APO). This is in accordance with the lack of cortico-striatal plasticity *in vitro* that recovers after DAergic treatment.[43] However, the four I-O curves were different in comparison to normal animals (i.e. the rightward shifted curve was the 10.00am curve). We hypothesized that due to the pharmacokinetic short-lasting properties of APO,[44] the recovery of the CS-EP is only incomplete. The post-synaptic action of APO could be another key factor for such a partial response.

Furthermore, the relationship between SWA and CS-EP strength was different across all groups. Indeed, naïve rats showed a strong positive correlation between changes of SWA power and of CS-EP V50 suggesting that urethane-induced SWA contributes to controlling both cortical and striatal excitability. In contrast, this type of correlation was completely lost in DA denervated animals and was not recovered by APO treatment. This suggests that only a physiological tonic DA substitution would completely restore the physiological modulation of CS-EP by SWA.

In agreement with our findings, the physiological corticostriatal plasticity strongly depends on DA system integrity and striatal DAergic denervation produces resilient biochemical and morphological changes.[12,45,46] The DA dependency of the urethane-induced SWA effect was attested to by the partial reversibility of the progressive reduction of synaptic efficacy obtained by APO treatment. The effect observed by DA-agonist treatment exhibits some parallels with the clinical observation of motor performance amelioration after sleep in PD patients featured by longer duration of disease and motor fluctuations.[16] Notably, PD patients with sleep benefit are more frequently under DA-agonist treatment. [16]

We emphasize that DA plays a crucial role for both physiological SWA activity and the homeostatic plasticity adjustment of the corticostriatal synapsis during sleep. This reinforces the need for an adequate DA replacement therapy during day and nighttime. In PD patients, the discontinuation of DA replacement therapy during the night could be responsible for aberrant synaptic changes that over the course of the disease could lead to pathophysiological changes such as observed in dyskinesia. [12,46]

We are aware of some limitations of the present study: 1) It lacks a comparable anesthetic not able to induce cortical SWA. However, as above mentioned, the isolated effect on delta power suggests an unrelated effect of the anesthetic but rather a specific SWA mediated effect. 2) We do not compare another dopaminergic drug, such as levodopa, in order to investigate putative pre-synaptic DA-mediated mechanism. 3) We do not assess the effect of dopaminergic treatment during the awake period in order to test a putative DA-mediated recovery of build-up synaptic strength.

Despite the above limitations, we can conclude that our results are in line with the hypothesized role of SWA on shaping synaptic strength over longer time scales. This is physiologically achieved during NREM sleep. Furthermore, our results also bear testimony to a fundamental role for physiological DA tone on homeostatic process, regulating SWA during the day and night which requires an adequate DAergic therapy.

Acknowledgments

We thank the *Fondazione per lo studio delle malattie neurodegenerative delle persone adulte e dell'anziano* for financial support to SG. This work was partially supported by a grant from the Baasch-Medicus Foundation to AKL and an IBRO-Swiss National Science Foundation grant to SW and AKL. The authors are particularly grateful to Sandra Pinton for her technical support and Dr Samantha Austen for the English revision of the manuscript.

Disclosure statement

The authors declare do not have any financial arrangements or connections pertinent to the submitted manuscript. Financial Disclosure: none.

Reference

1. Steriade, M., Timofeev, I. & Grenier, F. Natural waking and sleep states: a view from inside neocortical neurons. *J. Neurophysiol.* **85,** 1969–1985 (2001).
2. Steriade, M., Nuñez, A. & Amzica, F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J. Neurosci. Off. J. Soc. Neurosci.* **13,** 3252–3265 (1993).
3. Fox, K. & Armstrong-James, M. The role of the anterior intralaminar nuclei and N-methyl D-aspartate receptors in the generation of spontaneous bursts in rat neocortical neurones. *Exp. Brain Res.* **63,** 505–518 (1986).
4. Crook, J. & Lovick, T. Urodynamic function during sleep-like brain states in urethane anesthetized rats. *Neuroscience* **313,** 73–82 (2016).
5. Pagliardini, S., Gosgnach, S. & Dickson, C. T. Spontaneous sleep-like brain state alternations and breathing characteristics in urethane anesthetized mice. *PloS One* **8,** e70411 (2013).
6. Pagliardini, S., Funk, G. D. & Dickson, C. T. Breathing and brain state: urethane anesthesia as a model for natural sleep. *Respir. Physiol. Neurobiol.* **188,** 324–332 (2013).
7. Clement, E. A. *et al.* Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PloS One* **3,** e2004 (2008).
8. Galati, S. *et al.* The pharmacological blockade of medial forebrain bundle induces an acute pathological synchronization of the cortico-subthalamic nucleus-globus pallidus pathway. *J. Physiol.* **587,** 4405–4423 (2009).
9. Magill, P. J., Bolam, J. P. & Bevan, M. D. Dopamine regulates the impact of the cerebral cortex on the subthalamic nucleus-globus pallidus network. *Neuroscience* **106,** 313–330 (2001).

10. Tseng, K. Y. *et al.* Subthalamic nucleus lesions reduce low frequency oscillatory firing of substantia nigra pars reticulata neurons in a rat model of Parkinson's disease. *Brain Res.* **904,** (2001).
11. Hammond, C., Bergman, H. & Brown, P. Pathological synchronization in Parkinson's disease: networks, models and treatments. *Trends Neurosci.* **30,** 357–364 (2007).
12. Picconi, B. *et al.* Loss of bidirectional striatal synaptic plasticity in L-DOPA–induced dyskinesia. *Nat. Neurosci.* (2003). doi:10.1038/nn1040
13. Charpier, S. & Deniau, J. M. In vivo activity-dependent plasticity at cortico-striatal connections: evidence for physiological long-term potentiation. *Proc. Natl. Acad. Sci. U. S. A.* **94,** 7036–7040 (1997).
14. Poewe, W. & Hogl, B. Parkinson's disease and sleep. *Curr. Opin. Neurol.* **13,** 423–426 (2000).
15. Rye, D. B. & Jankovic, J. Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. *Neurology* **58,** 341–346 (2002).
16. Bateman, D. E., Levett, K. & Marsden, C. D. Sleep benefit in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **67,** 384–385 (1999).
17. Stefani, A. *et al.* Spontaneous sleep modulates the firing pattern of parkinsonian subthalamic nucleus. *Exp. Brain Res.* **168,** 277–280 (2006).
18. Galati, S. *et al.* Evidence of an association between sleep and levodopa-induced dyskinesia in an animal model of Parkinson's disease. *Neurobiol. Aging* **36,** 1577–1589 (2015).
19. Tononi, G. & Cirelli, C. Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. *Neuron* **81,** 12–34 (2014).
20. de Vivo, L. *et al.* Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science* **355,** 507–510 (2017).
21. Chauvette, S., Seigneur, J. & Timofeev, I. Sleep oscillations in the thalamocortical system induce long-term neuronal plasticity. *Neuron* **75,** 1105–1113 (2012).

22. Rall, W. Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.* **30,** 1138–1168 (1967).

23. Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U. & Tononi, G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat. Neurosci.* **11,** 200–208 (2008).

24. Nelson, S. B. & Turrigiano, G. G. Strength through Diversity. *Neuron* **60,** 477–482 (2008).

25. Olcese, U., Esser, S. K. & Tononi, G. Sleep and synaptic renormalization: a computational study. *J. Neurophysiol.* **104,** 3476–3493 (2010).

26. Czarnecki, A., Birtoli, B. & Ulrich, D. Cellular mechanisms of burst firing-mediated long-term depression in rat neocortical pyramidal cells. *J. Physiol.* **578,** 471–479 (2007).

27. Galiñanes, G. L., Braz, B. Y. & Murer, M. G. Origin and properties of striatal local field potential responses to cortical stimulation: temporal regulation by fast inhibitory connections. *PloS One* **6,** e28473 (2011).

28. Prosperetti, C., Di Giovanni, G., Stefani, A., Moller, J. C. & Galati, S. Acute nigro-striatal blockade alters cortico-striatal encoding: an in vivo electrophysiological study. *Exp. Neurol.* **247,** 730–736 (2013).

29. Borbély, A. A. & Achermann, P. Sleep homeostasis and models of sleep regulation. *J. Biol. Rhythms* **14,** 557–568 (1999).

30. Galati, S. *et al.* In vivo electrophysiology of dopamine-denervated striatum: focus on the nitric oxide/cGMP signaling pathway. *Synap. N. Y. N* **62,** 409–420 (2008).

31. Salvadè, A. *et al.* Distinct roles of cortical and pallidal β and γ frequencies in hemiparkinsonian and dyskinetic rats. *Exp. Neurol.* **275 Pt 1,** 199–208 (2016).

32. Galati, S. *et al.* Acute inactivation of the medial forebrain bundle imposes oscillations in the SNr: a challenge for the 6-OHDA model? *Exp. Neurol.* **225,** (2010).

33. Ryan, L. J., Tepper, J. M., Young, S. J. & Groves, P. M. Frontal cortex stimulation evoked neostriatal potentials in rats: Intracellular and extracellular analysis. *Brain Res. Bull.* **17,** 751–758 (1986).

34. Holst, S. C. *et al.* Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans. *J. Neurosci. Off. J. Soc. Neurosci.* **34,** 566–573 (2014).

35. Lobb, C. J. & Jaeger, D. Bursting activity of substantia nigra pars reticulata neurons in mouse parkinsonism in awake and anesthetized states. *Neurobiol. Dis.* **75,** 177–185 (2015).

36. Riedner, B. A. *et al.* Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep* **30,** 1643–1657 (2007).

37. Esser, S. K., Hill, S. L. & Tononi, G. Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. *Sleep* **30,** 1617–1630 (2007).

38. Hanlon, E. C., Faraguna, U., Vyazovskiy, V. V., Tononi, G. & Cirelli, C. Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. *Sleep* **32,** 719–729 (2009).

39. Perfetti, B. *et al.* Temporal evolution of oscillatory activity predicts performance in a choice-reaction time reaching task. *J. Neurophysiol.* **105,** 18–27 (2011).

40. Faraguna, U., Vyazovskiy, V. V., Nelson, A. B., Tononi, G. & Cirelli, C. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J. Neurosci. Off. J. Soc. Neurosci.* **28,** 4088–4095 (2008).

41. Morgante, F., Espay, A. J., Gunraj, C., Lang, A. E. & Chen, R. Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. *Brain J. Neurol.* **129,** 1059–1069 (2006).

42. Pritzel, M., Huston, J. P. & Sarter, M. Behavioral and neuronal reorganization after unilateral substantia nigra lesions: Evidence for increased interhemispheric nigrostriatal projections. *Neuroscience* **9,** 879–888 (1983).

43. Bagetta, V., Ghiglieri, V., Sgobio, C., Calabresi, P. & Picconi, B. Synaptic dysfunction in Parkinson's disease. *Biochem. Soc. Trans.* **38,** 493–497 (2010).

44. Hudson, J. L. *et al.* Correlation of apomorphine- and amphetamine-induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. *Brain Res.* **626,** 167–174 (1993).

45. Calabresi, P., Galletti, F., Saggese, E., Ghiglieri, V. & Picconi, B. Neuronal networks and synaptic plasticity in Parkinson's disease: beyond motor deficits. *Parkinsonism Relat. Disord.* **13 Suppl 3,** S259-262 (2007).

46. Calabresi, P., Filippo, M. D., Ghiglieri, V., Tambasco, N. & Picconi, B. Levodopa-induced dyskinesias in patients with Parkinson's disease: filling the bench-to-bedside gap. *Lancet Neurol.* **9,** 1106–1117 (2010).

Figures legends

Figure 1. Study protocol and histological assessment. A. Flow chart of the study. Three groups of animals (control, Parkinsonian and Parkinsonian-treated animals) were anesthetized with urethane and submitted every 2 hours to an input-output (I-O) stimulation with increased stimulation currents. B. Schematic representation of the stimulation protocol. A stimulation electrode was placed in the frontal right cortex. A deep striatal electrode was placed within the striatum for the cortico-striatal evoked potential, while a screw electrode was placed on the frontal left cortex for the recording of both ECoG and cortico-cortico evoked potential. C. Histological coronal section depicting the goodness of the TH lesion on the right side lesioned hemisphere. D. Representation of a recorded CS-EP. Field potential amplitude was measured between the N2 and P2 peak.

Figure 2. ECoG spectral analysis. A. The mean dominant frequency of the ECoG during all the 8 hours of recording. For all the animals (n = 16), we calculated the ECoG dominant frequency expressed during the time course of the urethane anaesthesia. The lines represent the mean dominant frequency in the control (turquoise, n = 6) and in Parkinsonian (red, n = 10). As shown both control and Parkinsonian expressed constantly a dominant frequency below 3 Hz within the delta SWA range. B. The total power of the delta frequency range (0.1-4 Hz) calculated for 10 minutes before each four I-O curves (10.00am; 12.00pm; 02.00pm; 04.00pm). We found a significant decreases a progressive reduction of the delta total power between 10.00am and the following hours (12.00pm; 02.00pm; 04.00pm) only in the control animals. We did not observed significant changes in both 6-OHDA and 6-OHDA APO treated rats although the latter show a tendency to have higher values at 10.00 am and 12.00 pm.

C. The total power of the delta frequency range (0.1-4 Hz) calculated for 10 minutes before each four I-O curves (10.00am; 12.00pm; 02.00pm; 04.00pm). We observed a significant difference only at the end of the awake period (10.00am) in normal animals.

Data are expressed as mean±SEM; * = $P < 0.05$; n.s. = not significant; § = $P < 0.001$ Control vs 6-OHDA and Control vs 6-OHDA+APO

Figure 3. Normalized evoked response amplitude in control animals (n = 6). A. The I-O curve of CC-EP amplitude performed in the four tested hours (10.00am; 12.00pm; 02.00pm; 04.00pm). The stimulus strength necessary to give the 50% response (V50) was lower in the I-O curve performed at 10.00 am (pink line) in comparison with the other I-O curves. While the V50 higher value was obtained with the last I-O curve (blue line). B. Similarly, the I-O curves of the CS-EP showing a significant different curve fitting for the four hours in which the first (10.00am, pink line) has the lower V50 value while the last (4.00pm, blue line) has the highest one.
V50 = the stimulus strength necessary to give the 50% response; data are expressed as mean

Figure 4. Normalized evoked response in 6-OHDA animals (n = 5) and Parkinsonian treated with APO (n =5). A. The curve fitting of the I-O curve of the CS-EP amplitude was not significant different between the four hours. A shared curve fit is showed on the plot. B. The Parkinsonian animals treated with APO showed a significant different curve fit of the I-O curve amplitude. However, the first I-O curve performed on the morning (10.00am) showed the highest V50 level, i.e. the lasses excitability. The lower V50 was observed at 02.00pm when three APO challenge have been performed.
V50 = the stimulus strength necessary to give the 50% response; data are expressed as mean

Figure 5. Evoked response in the three groups of animals at the four examined hours. The maximal V50 difference between groups was observed at 10.00am with a progressive reduction between control and 6-OHDA animals up to become not significant at 4.00pm in which a shared black curve fit is shown.

Figure 6. Correlation analysis between changes of SWA total power and V50 measured at 10.00am and 4.00pm. Only in control, we observed a statistical significance of Pearson correlation with an $R^2 = 0.835$. Whilst no correlation has been observed in the other two groups of animals.